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Nitrification inhibitors affect the phosphorus release capacity by phosphate-solubilizing fungi

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Abstract: The 3,4-dimethyl pyrazole phosphate (DMPP) has been applied in long-scale agricultural production. This research investigated the phosphorus (P) release capacity by two types of phosphate solubilizing fungi (PSF) *Aspergillus niger* and *Penicillium oxalicum* under different DMPP concentrations. The 10 and 20 mg/L DMPP can enhance the P release capacity in two PSF *Aspergillus niger* and *Penicillium oxalicum*, respectively. Both the P release capacity in these two fungi was limited in a higher DMPP concentration (>20 mg/L). Oxalic and citric acids were the primary organic acids secreted by *Aspergillus niger* and *Penicillium oxalicum* under different DMPP concentrations, respectively. Meanwhile, DMPP can also affect the pyruvate dehydrogenase enzyme activity in these two fungi. This research indicated that the use of DMPP should be more attention to PSF and P release in soil.

Keywords: phosphate solubilizing fungi; nitrification inhibitor; phosphorus release; organic acids; TCA cycle

1. Introduction

Phosphate-solubilizing fungi (PSF) can promote the release of phosphorus (P) from insoluble phosphate in soil via the secretion of organic acid [1]. The organic acids (OA), *e.g.*, oxalic and citric produced by PSF are the primary pathway for the P release [2]. These OA can promote P release via acidification and complex with metal cations [3]. In addition, PSF can also obtain nutrients from root exudates and root exfoliations, and transfer insoluble P to

available P for plant utilization [4]. *Penicillium oxalicum* (*P. oxalicum*) and *Aspergillus niger* (*A. niger*) are the common PSF in soil [5]. Due to *P. oxalicum* and *A. niger* having a high ability in organic acid secretion, hence usually applied in insoluble phosphate dissolution[6]. The tricarboxylic acid cycle is the primary pathway for organic acid production by PSF [7]. Organic acid secretion by PSF is usually affected by pH, carbon, nitrogen and phosphate resources, and other environmental factors [8,9]. For example, when pH is 6.5, it can promote *P. oxalicum* to secrete more formic acid, and enhanced *A. niger* produce more oxalic acid [10]. In the study of *penicillium aurantiogriseum* (*P. aurantiogriseum*), oxalic acid is more secretion occurred in glucose, and citric acid is more secretion occurred in sucrose [11]. In addition, different nitrogen sources also affect the OA secretion by PSF. Nitrate can significantly promote the production of oxalic acid by *A. niger* than ammonium and urea [12]. Meanwhile, the different types of phosphate would also affect the production of OA by PSF. Ferric phosphate (Fe-P) can stimulate *A. niger* to produce more citric acid, while tricalcium phosphate (Ca-P) dominates the production of oxalic acid [13].

Nitrification inhibitor (NI) can inhibit nitrosamines and other activities, which has been applied in agricultural production. The application of NI can delay the transformation of ammonium to nitrate nitrogen in soil, reducing nitrogen leaching loss and N_2O emission [14]. In addition, the use of NI can also significantly improve nitrogen fertilizer efficient and crop yield, and reduce the pollution of groundwater. In the past, the most used NI was 2-chloro-6-(trichloromethyl) pyridine (CP) and dicyandiamide (DCD). However, CP and DCD take the risk of large applications and harm to soil health. Therefore, a new NI of 3, 4-dimethyl pyrazole phosphate (DMPP) was developed and applied [15]. Compared with DCD and CP, DMPP is more efficient and safe in application. At present, DMPP has been widely used in agricultural production.

DMPP can inhibit ammonium transfer to nitrate via limit the growth of ammonia-oxidizing bacteria (AOB) [16]. However, the research on DMPP mainly focuses on microorganisms related to the nitrogen cycle, but the impact on PSF is not clear. In this study, the P release capacity in two PSF *Aspergillus niger* and *Penicillium oxalicum* were investigated under different DMPP concentrations. The inductively coupled plasma emission spectrometer (ICP-OES) can analyze the concentration of soluble P. The organic acids produced by *Aspergillus niger* and *Penicillium oxalicum* were analyzed by HPLC.

The enzyme activity of pyruvate dehydrogenase (PDH) was also determined.

2. Materials and methods

2.1. Phosphate rock and PSF preparation

Phosphate rock fluorapatite (FAp) was collected from the Nanchong phosphate mine (30.26 N, 116.04 E) in Anqing city, Anhui Province, China. The collected FAp was firstly ground into powder, and then filtered through an 80-μm mesh sieve before the experiment [17]. DMPP was supplied by Shanghai Macklin Biochemical Co., Shanghai, China.

The PSF of *Aspergillus niger* (AH-F-1-2, CGMCC No. 23272) and *Penicillium oxalicum* (AH-F-2-7, CGMCC No. 22475) were isolated from soybean rhizosphere at the

Suzhou City, Anhui Province, China. *Penicillium oxalicum* and *Aspergillus niger* were cultured in a Potato Dextrose Agar (PDA) medium for five days at 28 ℃. The formed spores were collected by using sterile water. Then, filter the mycelium with sterilized three layers gauze to obtain the suspensions of fungal spore. The collected fungal spore concentration was diluted to 10^7 CFU/mL by using a hemocytometer [18].

2.2. Fungal incubation under different DMPP concentrations

The concentrations of DMPP in this research have five treatments, *i.e.*, 0, 10, 20, 50 and 100 mg/L. Before the incubation, a 250 mL conical flask was sterilized for 20 minutes at 121 ℃ with contained a 100 mL Pikovskaya (PVK) medium [16]. PVK contained 10 g glucose, 0.2 g KCl, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.03 g $MnSO_4 \cdot 4H_2O$, and 0.2 g NaCl in 1 L deionized water. Then, 0.5 g FAp with 1 mL of *P. oxalicum* (POX) and *A. niger* (ANG) spores were respectively added to the 250 mL flask. Finally, a parafilm (BS-QM-003, Biosharp) contained a 0.22 μm air-breathable membrane in the middle was used in the conical flask [4]. After seven days of incubation at 180 rpm and 28 ℃, the PVK medium was filtered by using a 0.22 μm polyethersulfone (PES) membrane [3]. The filtrates were collected to measure organic acid, pH value and P concentration. The collected sediment was dried at 65 ℃ for 24 hours for analysis of dry biomass [4]. All treatments were repeated three times.

2.3. Instrumentation

The P concentration in the medium was analyzed by ICP-OES (iCAP 7000 series, Thermo Fisher Scientific, USA). The calibration curves of P (0, 10, 50, 100, 200 and 500 mg/L) were prepared by using the P standard. HPLC (Agilent 1260) was used to measure the concentration of organic acids.

3. Results and discussion

3.1. Fungal biomass and pH

After incubation for seven days, the fungal dry biomass in POX treatment ranged from 0.35 to 0.36 g in the different DMPP concentrations, respectively (Figure 1). In ANG treatment, the fungal dry biomass was 0.38 g, 0.37 g, 0.38 g, 0.36 g and 0.35 g under different DMPP concentration after incubation for seven days, respectively (Figure 1). The fungal dry biomass between *P. oxalicum* and *A. niger* was similar under different DMPP concentration. The different DMPP concentrations did not affect the growth of PSF.

The initial pH of the PVK medium was 7.0. In POX treatment, the pH value was 4.59, 4.22, 3.62, 4.96 and 5.25 after incubation for seven days, respectively (Figure 2). *P. oxalicum* has the lowest pH value in 20 mg/L DMPP concentration (3.62). In ANG treatment, all of the pH value was lower than in POX treatment, *i.e.*, 3.56, 3.46, 3.54, 3.59 and 3.46, respectively (Figure 2). The low pH value in POX and ANG treatment indicated that these two fungi can secrete organic acid under different DMPP concentration. However, the lowest pH value in POX occurred in 20 mg/L DMPP, while in ANG was 10 mg/L DMPP

concentration (Figure 2). The low concentration of DMPP (< 20 mg/L) could stimulate organic acid production by *P. oxalicum* and *A. niger*. However, *A. niger* has a lower inhibit DMPP concentration in organic acid secretion.

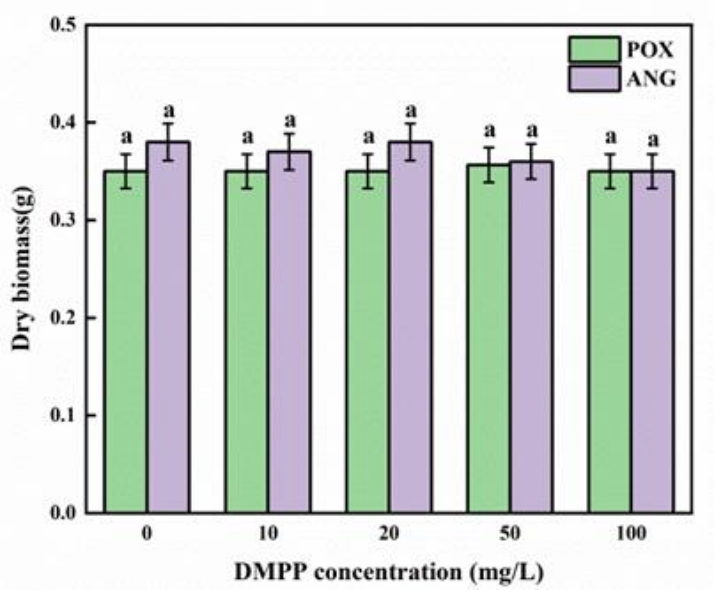

Figure 1. The dry biomass in each treatment after incubation for seven days. POX: *P. oxalicum*. ANG: *A. niger*.

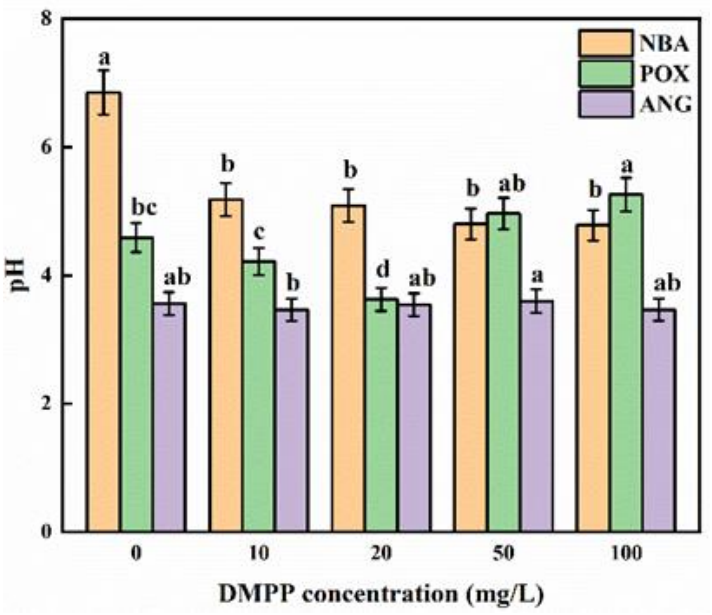

Figure 2. The pH in each treatment after incubation for seven days. NBA: DMPP without PSF. POX: *P. oxalicum*. ANG: *A. niger*.

3.2. P concentration and organic acid secretion

The P content in POX treatment was increased from 265.40 to 293.36 mg/L at the DMPP concentration of less than 20 mg/L (Figure 3). However, the high DMPP concentration (50 and 100 mg/L) limited the P release from FAp. The P content in 50 and 100 mg/L DMPP concentration decreased to 188.36 and 182.18 mg/L (Figure 3) similar trends were also observed in ANG treatment. The highest P content in ANG treatment occurred in 10 mg/L DMPP concentration, *i.e.*, 220.97 mg/L (Figure 3). When the DMPP concentration was higher than 20 mg/L, the P content in ANG treatment decreased to 175.37, 178.22 and 181.91 mg/L, respectively (Figure 3). Low concentration of DMPP can improve the P release from FAp by PSF, while the high concentration would inhibit this process.

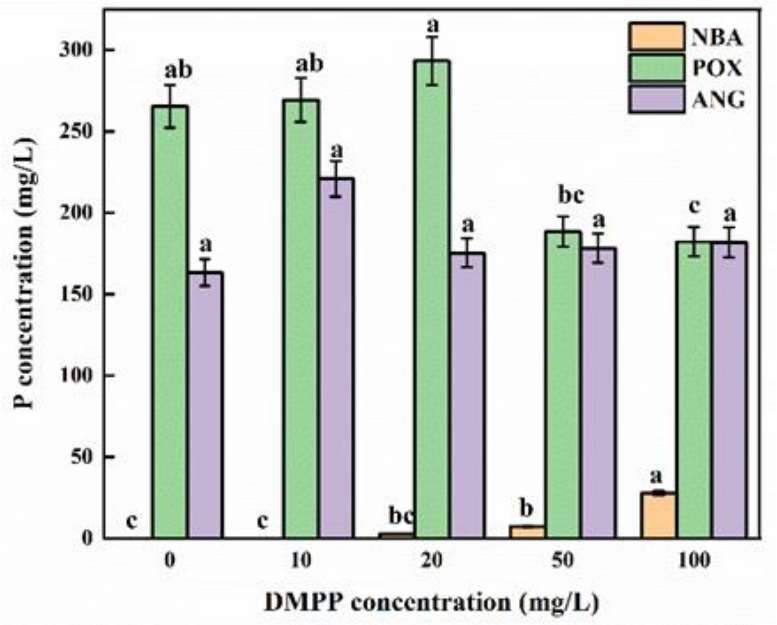

Figure 3. The concentrations of P in each treatment after incubation for seven days. NBA: DMPP without PSF. POX: *P. oxalicum*. ANG: *A. niger*.

The oxalic acid concentration in POX treatment without DMPP was 43.04mg/L after incubation for seven days (Figure 4). However, the increased DMPP concentration significantly decreased the secretion of oxalic acid lower than 30 mg/L by *P. oxalicum* (Figure 4). Compared with *P. oxalicum*, *A. niger* has a higher secretion of oxalic acid. In ANG treatment, the highest oxalic acid concentration occurred in 10 mg/L DMPP, *i.e.*, 90.85 mg/L (Figure 4). However, the oxalic acid concentration in ANG treatment decreased to 69.61, 61.43 and 59.72 mg/L with the DMPP concentration increased to 20, 50 and 100 mg/L, respectively (Figure 4). Oxalic acid is the primary organic acid secreted by *A. niger* [13], and the DMPP can affect the secretion of oxalic acid.

Citric acid was the primary organic acid secreted by *P. oxalicum*. The concentration of citric acid in POX was 200.79 mg/L, 217.04 mg/L, 220.36 mg/L, 190.45 mg/L and 145.49 mg/L in different DMPP concentrations, respectively (Figure 5). In ANG treatment, the citric acid concentration in 0 mg/L DMPP was 89.24mg/L. However, the increased DMPP

concentration decreased the production of citric acid by *A. niger*. After incubation for seven days, all of the citric acid concentration was lower than 60 mg/L in different DMPP concentrations (Figure 5). DMPP inhibited the production of citric acid by *A. niger* and stimulated critic acid production in *P. oxalicum*. It is worth noting that the low concentration of DMPP (< 20 mg/L) can promote the production of citric acid by *P. oxalicum*.

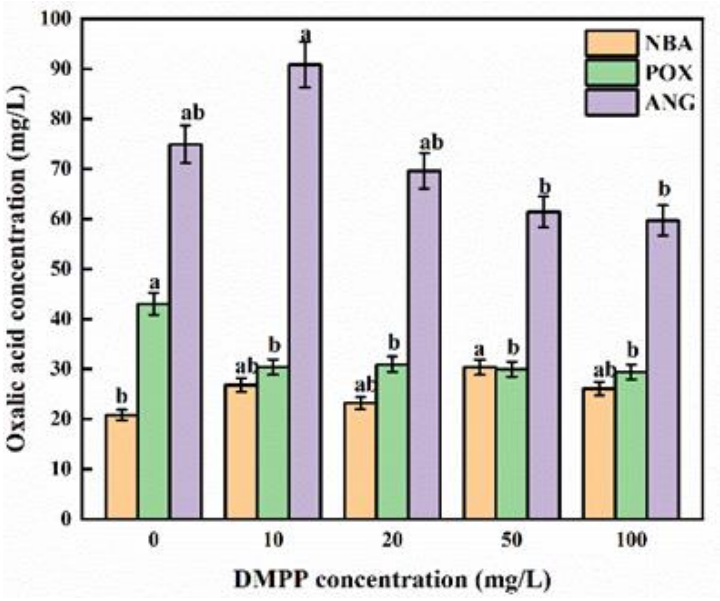

Figure 4. The oxalic acid concentration in each treatment after incubation for seven days. NBA: DMPP without PSF. POX: *P. oxalicum*. ANG: *A. niger*.

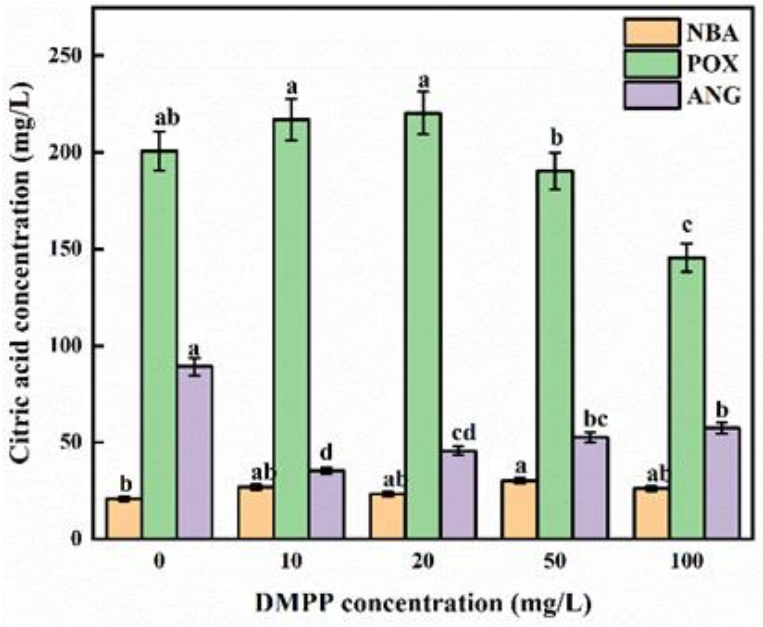

Figure 5. The citric acid concentration in each treatment after incubation for seven days. NBA: DMPP without PSF. POX: P. oxalicum. ANG: A. niger.

3.3. The enzyme activity

The PDH enzyme activity in POX treatment was 1.48 nmol/min/g, 2.47 nmol/min/g, 3.26 nmol/min/g, 2.04 nmol/min/g, and 1.77 nmol/min/g after incubation for seven days, respectively (Figure 6). In ANG treatment, the PDH enzyme activity was 1.62 nmol/min/g, 2.35 nmol/min/g, 1.87 nmol/min/g, 1.28 nmol/min/g, and 1.10 nmol/min/g, respectively (Figure 6). The PDH enzyme activity has the similar trend with oxalic acid concentration in ANG and citric acid concentration in POX under different DMPP concentration. PDH is the key rate-limiting enzyme of the tricarboxylic acid cycle and regulates the secretion of organic acids by PSF [13]. Both oxalic and citric acid are intermediate products of the tricarboxylic acid cycle. DMPP can regulate the P release capacity *A. niger* and *P. oxalicum* via the tricarboxylic acid cycle. In addition, the synthesis of PDH was regulated by the related gene expression, hence it is worth further research in gene level.

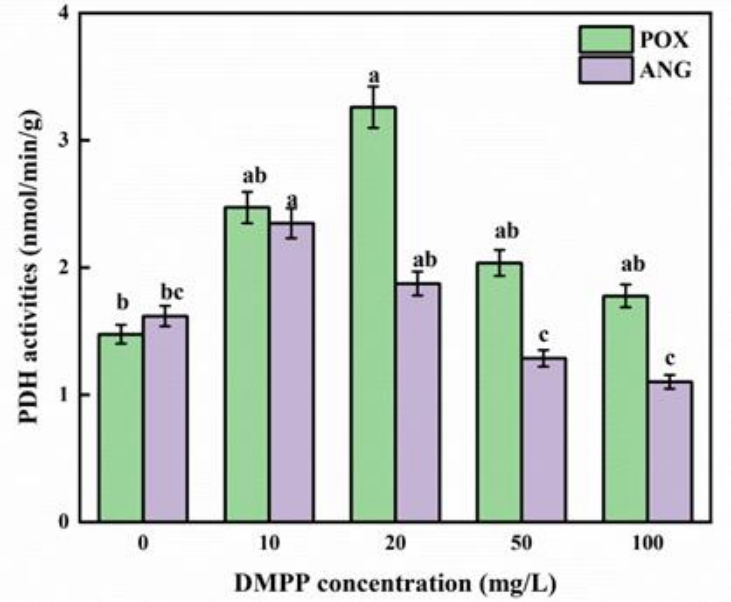

Figure 6. The pyruvate dehydrogenase activities in each treatment after incubation for seven days. POX: *P. oxalicum*. ANG: *A. niger*.

4. Conclusion

This study investigated the P release capacity of two typical PSF under different DMPP concentrations. *A. niger* and *P. oxalicum* have the highest P release capacity in 10 and 20 mg/L DMPP concentrations. More than 20 mg/L DMPP can inhibit the FAp dissolution and P release by *A. niger* and *P. oxalicum*. Oxalic acid is the primary organic acid produced by *A. niger*, while *P. oxalicum* dominates the production of citric acid. The different DMPP concentrations can affect the production of organic acid by PSF via the tricarboxylic acid cycle. DMPP is the commonly used biological agents in agricultural production, these results indicate that the use of DMPP has a potential influence in soil available P via change the P release capacity of PSF.

Acknowledgments

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Conflicts of interests

The authors declare no conflicts of interests.

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